

Antigen-binding units

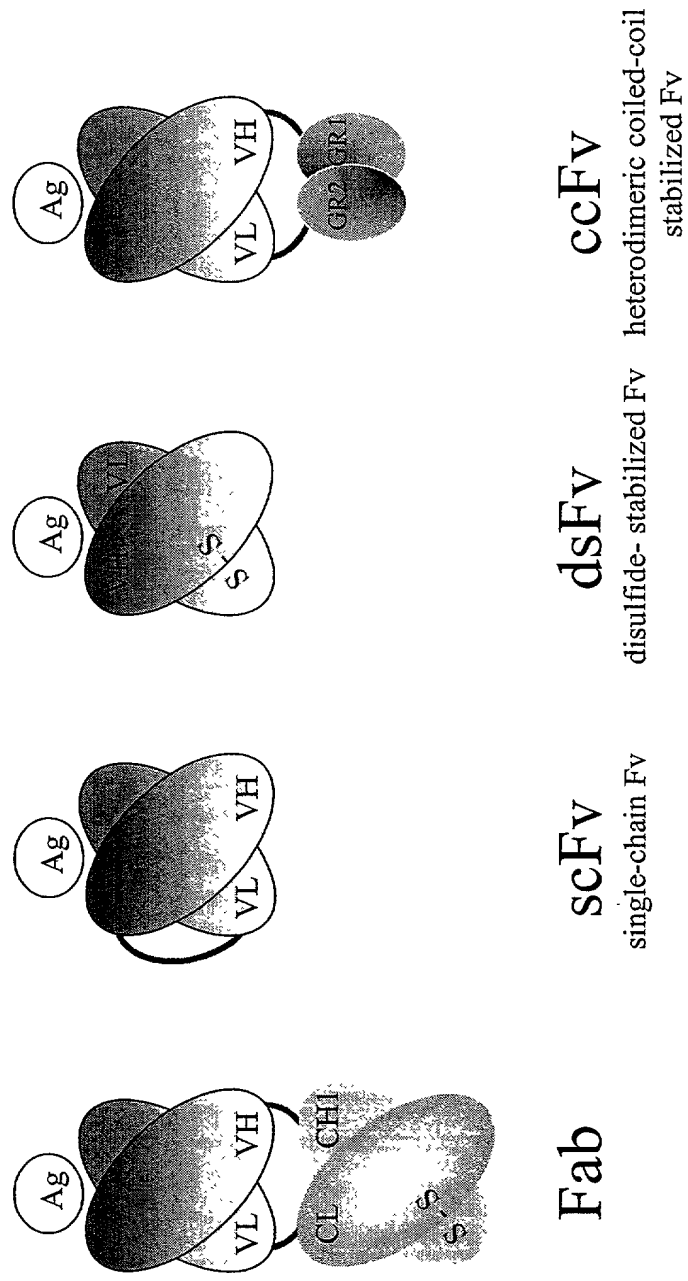


Fig. 1

Sequences of coiled-coil domain in ccFv

GR1 Sequence Range: 1 to 146

XbaI 10 20 30 40 50
 TCTAGAGGTGGAGGAGGTGAGGAGAACTCCCGGCTGTTGGAGAAAGGAGAA
S R G G G G G E E E K S R L L E K E N 100
 60 70 80 90
 CCGTGAAC TGAAAGATCAT TGTGAGAAAGAGGCGTGTCTCTGAAC
 R E L E K I I A E K E E R V S E
 110 120 130 140 ASCII
 TGGCGCATCAACTCCAGTCTGTAGGAGGTTGTTAATAGGGCGGCC
 L R H Q L Q S V G G C * *

GR2 Sequence Range: 1 to 140

XhoI 10 20 30 40 50
TCTCAGAGGTGTTGAACATCCCGCCTGGAGGGCTACAGTCAGAAAA
S R G G G G T S R L E G L Q S E N
60 70 80 90 100
CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGG
H R L R M K I T E L D K D L E E
110 120 130 NotI 140
TCACCATGCAGCTGCAGGACGTCGGAGGTTGCGGGCGCGC
V T M Q L Q D V G G C A A A

Fig. 2

Vectors for antibody expression

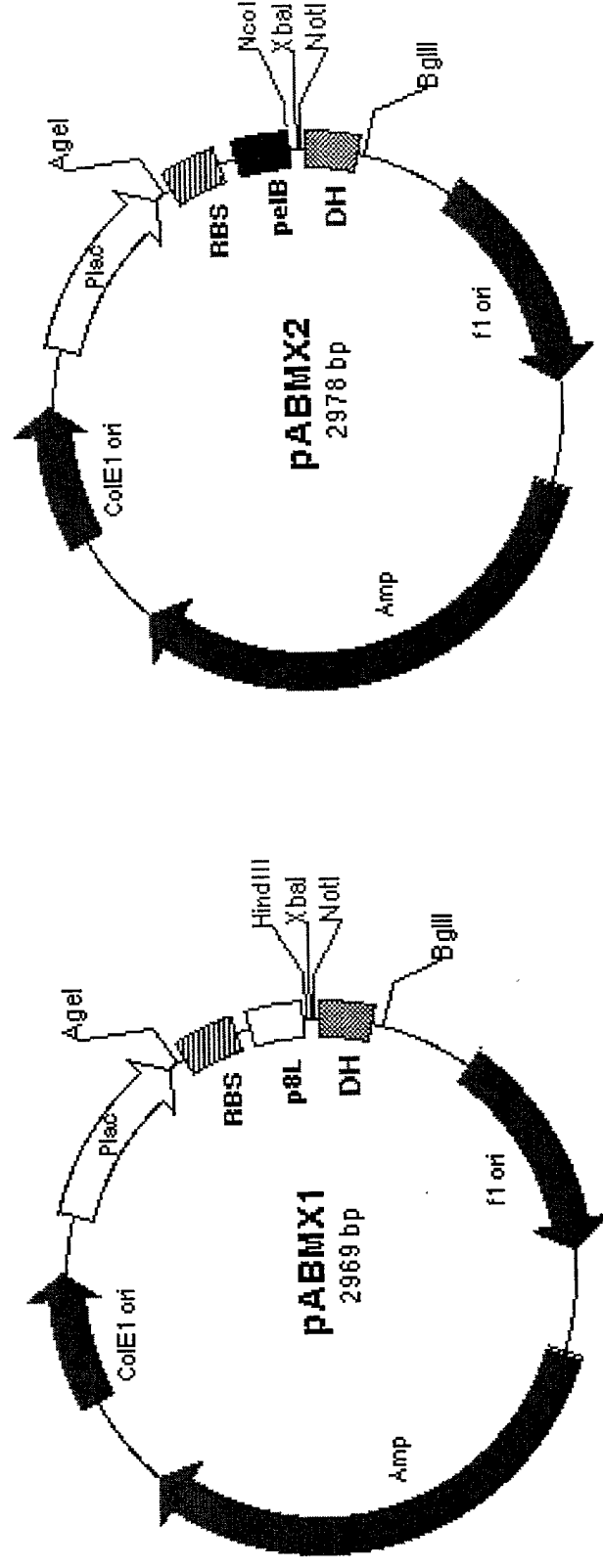


Fig. 3A

PABMX1 vector: sequence from AgeI to BglII

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
P8 Leader
ATGAAAAAGTCTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA XbaI
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT BglII
A A A Y P Y D V P D Y A G G H H H H H * R S

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PABMX2 vector: sequence from AgeI to BglII

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
pelB Leader
ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCGCCAGCCCGCCCTGCAGGCCCTCTAGA XbaI
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
NotI
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT BglII
A A A Y P Y D V P D Y A G G H H H H H * R S

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Fig. 3B

Vectors for antibody display

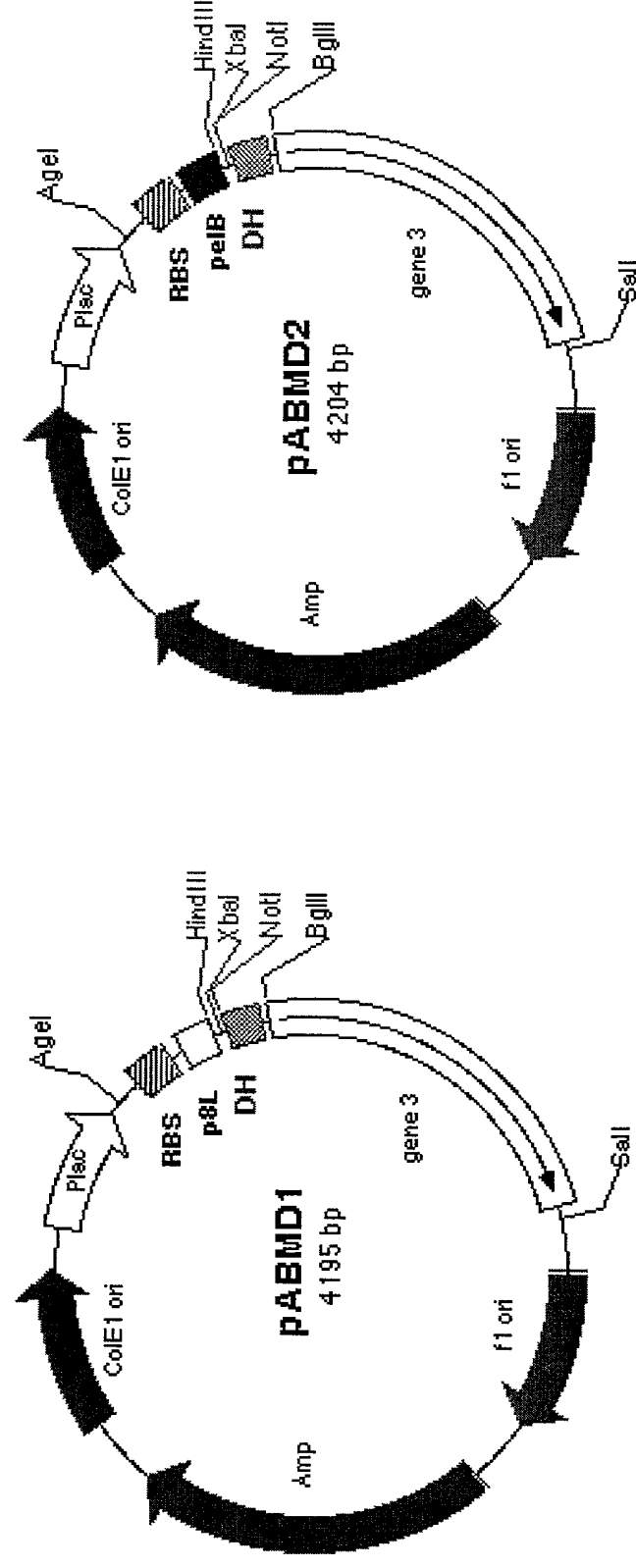


Fig. 4A

PABMD1 vector: sequence from AgeI to Sall

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCCTT TTAACCTTTAG TAAGGAGG AATTAAAAA
P8 Leader
ATGAAAAAGTCCTTAGTCCTCAAAAGCCTCCGTAGCCGTTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA
M K K S L V L K A S V A V A T L V P M L S F A S R
NotI
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
HA-tag
Gene 3
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ----- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ----- A N I L R N K E S *
Sall

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PABMD2 vector: sequence from AgeI to Sall

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lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCCTT TTAACCTTTAG TAAGGAGG AATTAAAAA
pelB Leader
ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCCCTGCAGGCCCTCTAGA
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
NotI
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
HA-tag
Gene 3
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ----- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ----- A N I L R N K E S *
Sall

```

Fig. 4B

Vectors for ccFv expression

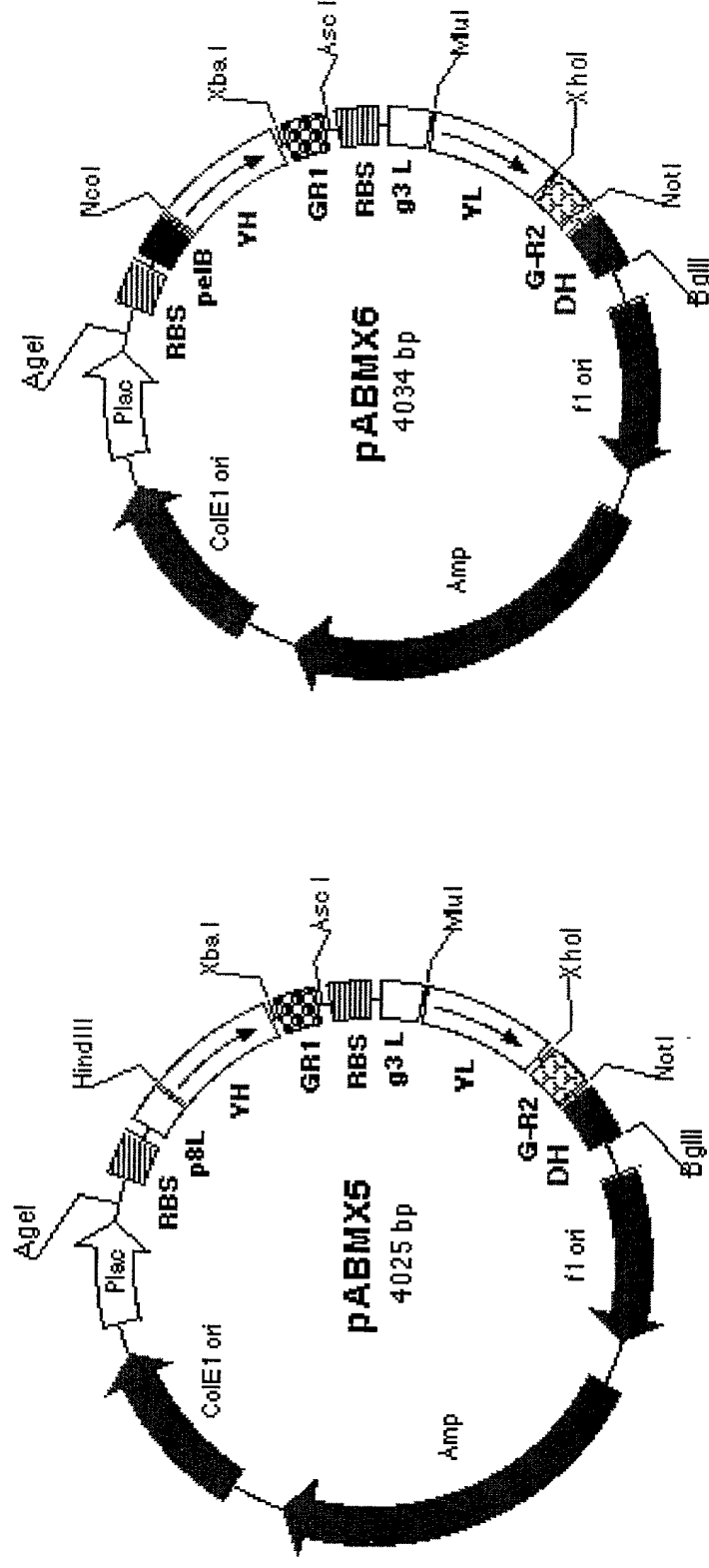


Fig. 5A

PABMX5 vector: sequence from p8 leader to DH-tag

P8 Leader
 ATGAAAAAGTCTTTAGTCTCTCAAGCCTCCGTAGCCGTGCTACCTCGTTCCGATGCTAAGCTTCGCT VH XbaI
 M K K S L V L K A S V A V A T L V P M L S F A S R
 Ascl GR1 GGCGCGCCACAATTTACAGTAAGGAGGTTTAACTT ATGAAAAAAATTATTATTCGCAATTCCCTTTAGTTGTTCTCT
 S/D VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA
 MluI XhoI NotI HA-tag
 TTCTATTCTCACTCCGCTACGCGT VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA
 F Y S H S A T R S R A A A Y P Y D V P D Y A
 His-tag
 GGAGGT CATCACCATCATCACCAT TAG
 G G H H H H H H *

PABMX6 vector: sequence from pelB leader to DH-tag

pelB Leader
 ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCGCATGGCG VH XbaI
 M K Y L L P T A A A G L L L L A A Q P A M A S R
 Nco I
 Ascl GR1 GGCGCGCCACAATTTACAGTAAGGAGGTTTAACTT ATGAAAAAAATTATTATTCGCAATTCCCTTTAGTTGTTCTCT
 S/D VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA
 MluI XhoI NotI HA-tag
 TTCTATTCTCACTCCGCTACGCGT VL TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACGACTACGCA
 F Y S H S A T R S R A A A Y P Y D V P D Y A
 His-tag
 GGAGGT CATCACCATCATCACCAT TAG
 G G H H H H H H *

Fig. 5B

Vectors for ccFv display

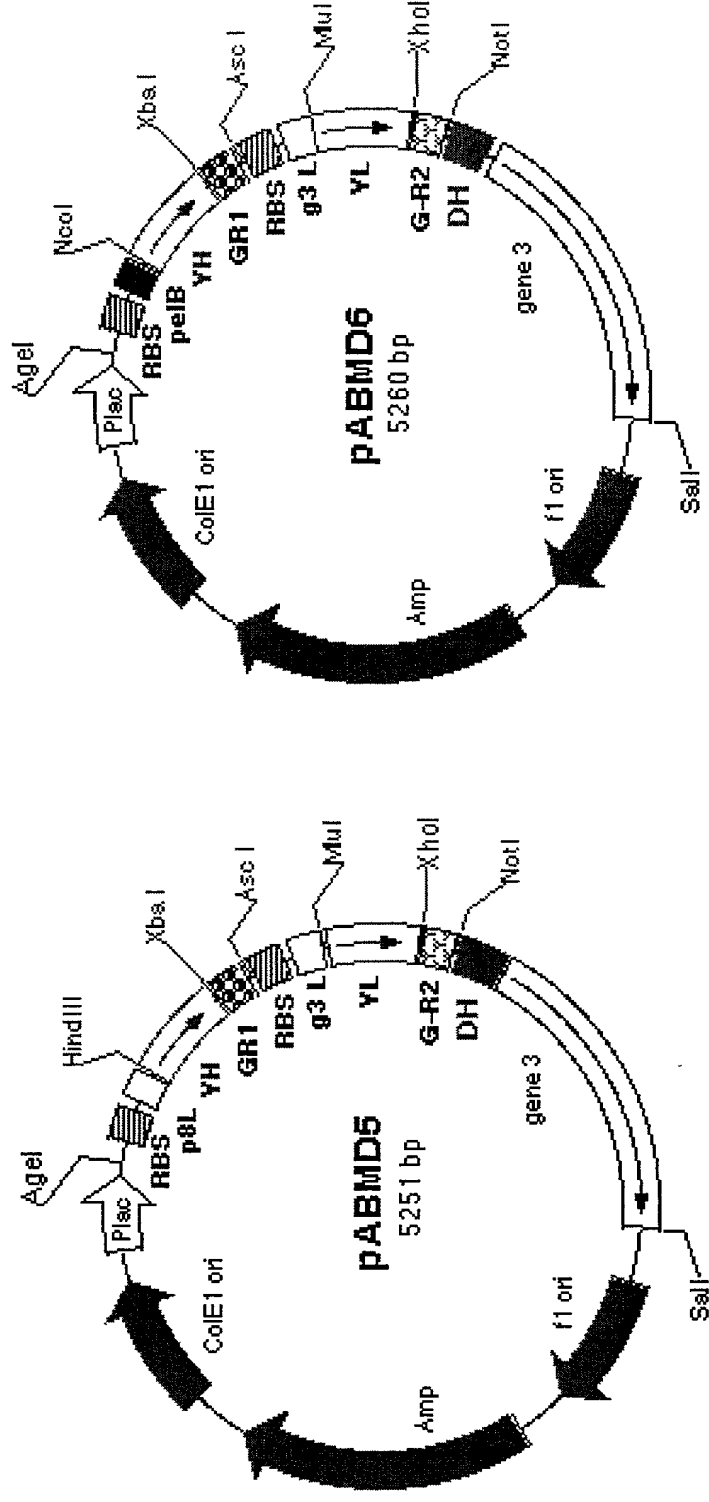


Fig. 6A

PABMD5 vector: sequence from HindIII to SalI

P8 Leader
 ATGAAAAAGTCTTAGTCTCTCAAGCCTCCGTAGCCGTGCTACCCCTCGTTCCGATGCTAAGCTTCGCT VH XbaI
 M K K S L V L K A S V A T L V P M L S F A S R

AscI
GR1 GCGCGCCACAATTTCACAGTAAGGAGGTTAACTT ATGAAAAAATATTATTTCGCAATTCCTTTAGTTGTTCTCT
 M K K L L F A I P L V P

S/D
 P3 Leader
 HA-tag
 NotI
 MluI XhoI TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACCAGACTACGCA
 TTCTATTCTCACTCCGCTACGCGT VL S R A A Y P Y D V P D Y A
 F Y S H S A T R His-tag SalI

Gene 3
 GGAGGT CATCACCATCATCACCAT TAG GGAGGCGGT ACTGTTGAAAGTTGT---CTGCGTAATAAGGAGTCTTAA GTGAC
 G G H H H H H * G G G T V E S C --- L R N K E S *

PABMX6 vector: sequence from pelB leader to DH-tag

pelB Leader
 ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCCATGGCG VH XbaI
 M K Y L L P T A A A G L L L L A A Q P A M A S R

AscI
GR1 GCGCGCCACAATTTCACAGTAAGGAGGTTTAACTT ATGAAAAAATATTATTTCGCAATTCCTTTAGTTGTTCTCT
 M K K L L F A I P L V P

S/D
 P3 Leader
 HA-tag
 NotI
 MluI XhoI TCTCGA GR2 GCGGCCGCTTATCCATACGACGTACCAGACTACGCA
 TTCTATTCTCACTCCGCTACGCGT VL S R A A Y P Y D V P D Y A
 F Y S H S A T R His-tag SalI

Gene 3
 GGAGGT CATCACCATCATCACCAT TAG GGAGGCGGT ACTGTTGAAAGTTGT---CTGCGTAATAAGGAGTCTTAA GTGAC
 G G H H H H H * G G G T V E S C --- L R N K E S *

Fig. 6B

Vector for ccFv expression in Yeast

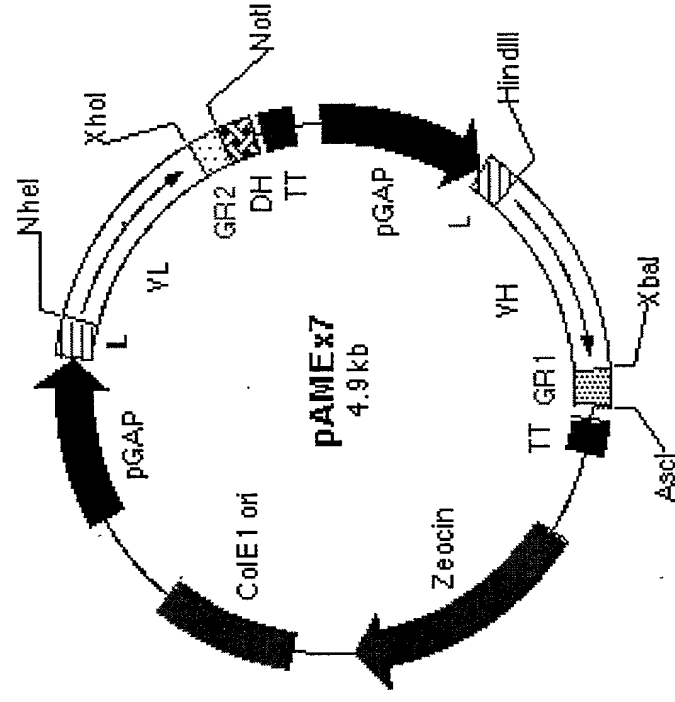


Fig. 7

Soluble antibody ELISA

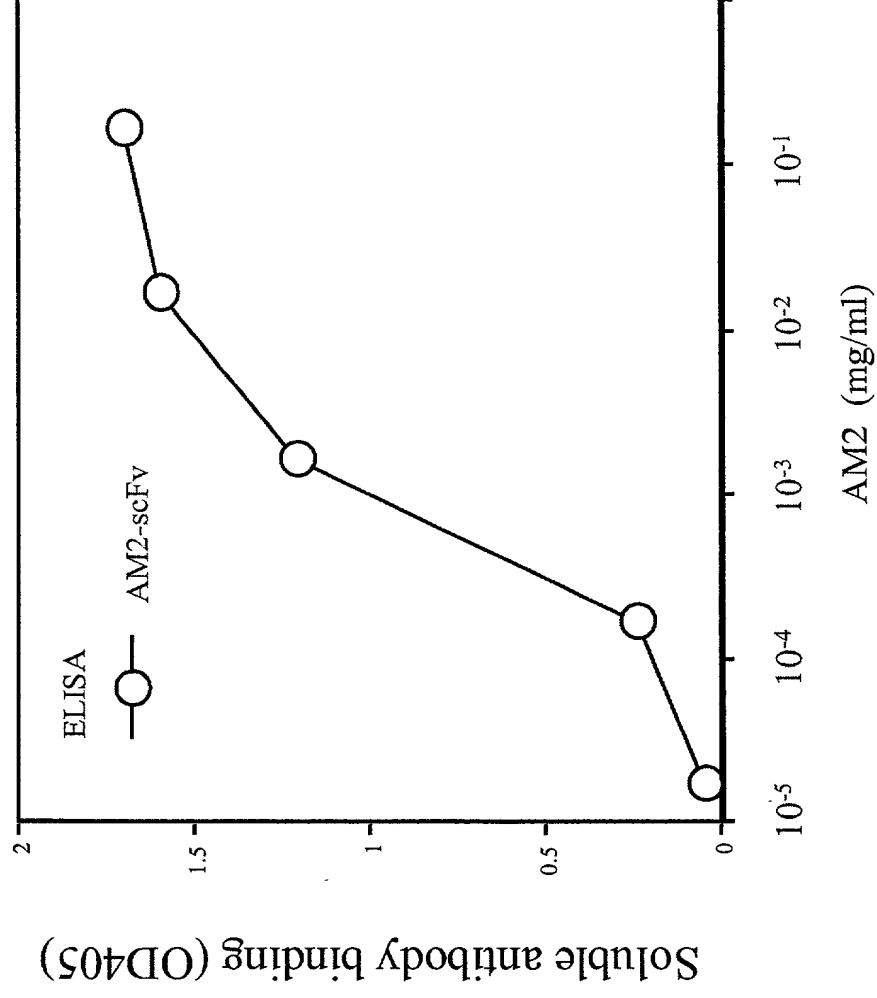


Fig. 8

AM2-scFv display on phage by pABMD1 vector

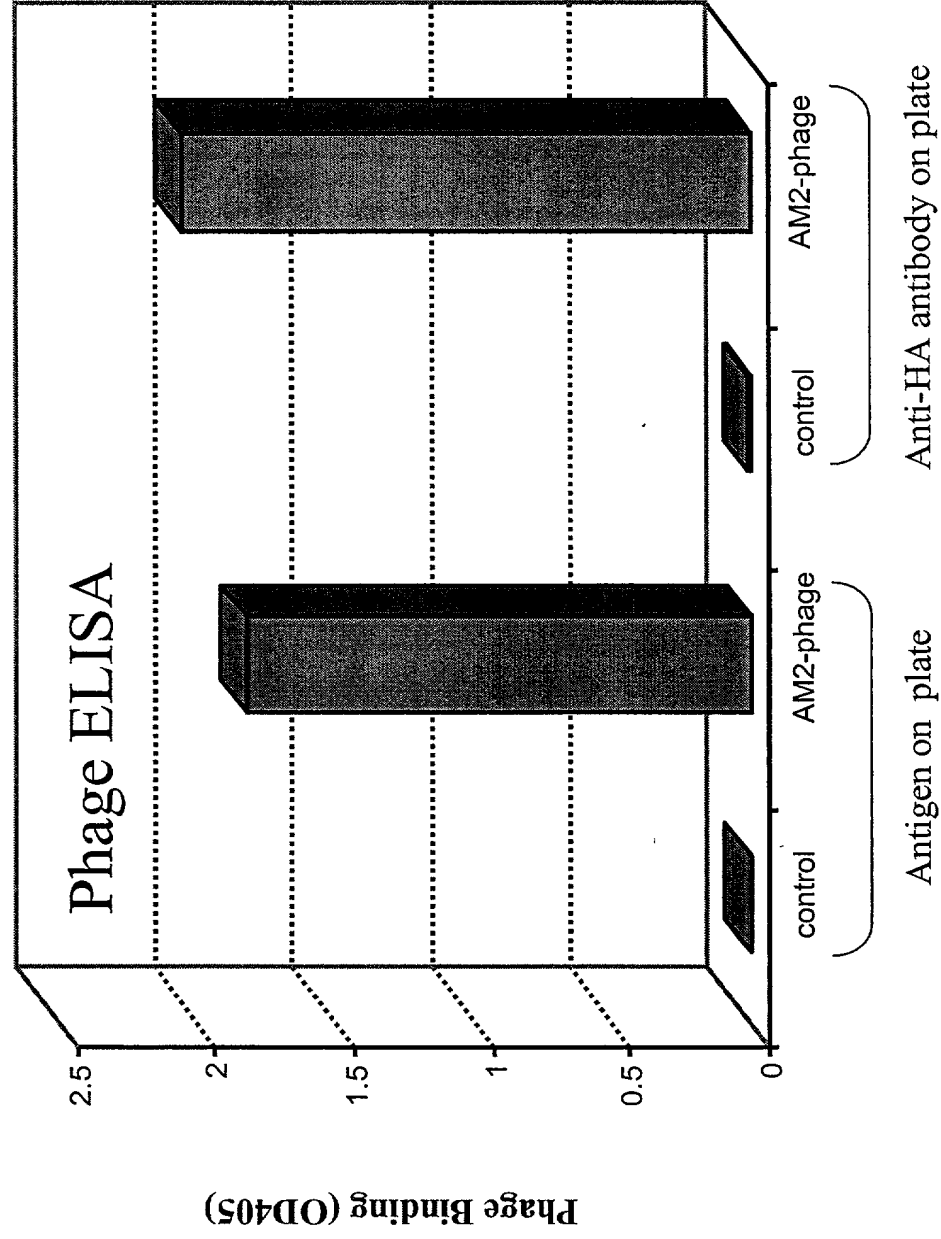


Fig. 9

Soluble AM1-ccFv antibody expression in *E. coli*

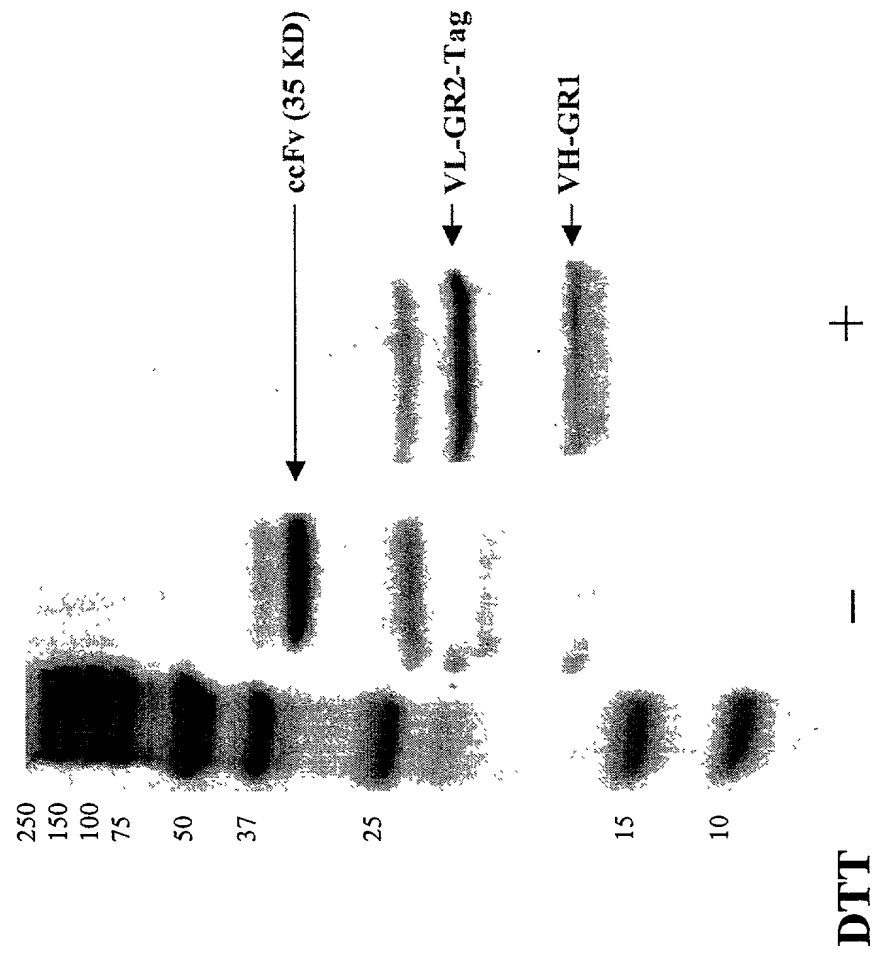


Fig. 10A

Soluble AM1-ccFv antibody binds to antigen

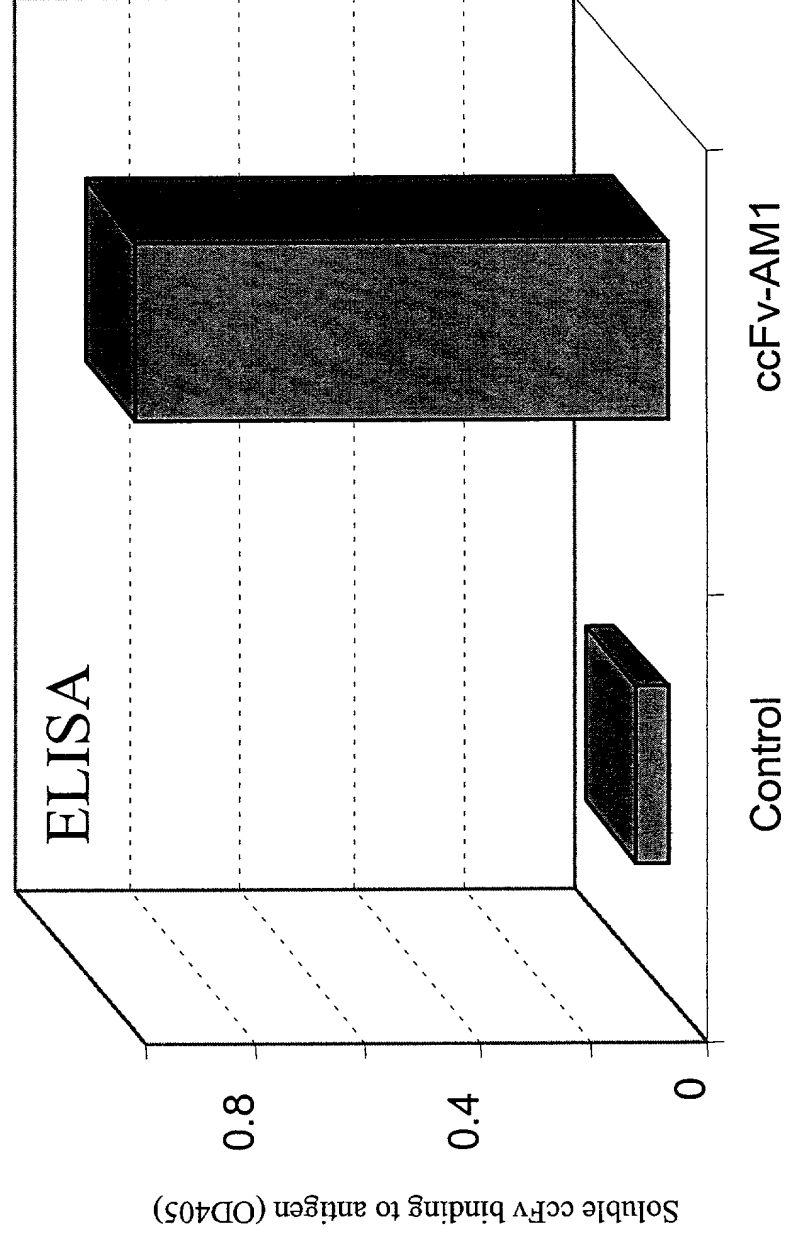


Fig. 10B

AM1-ccFv Antibody display on phage

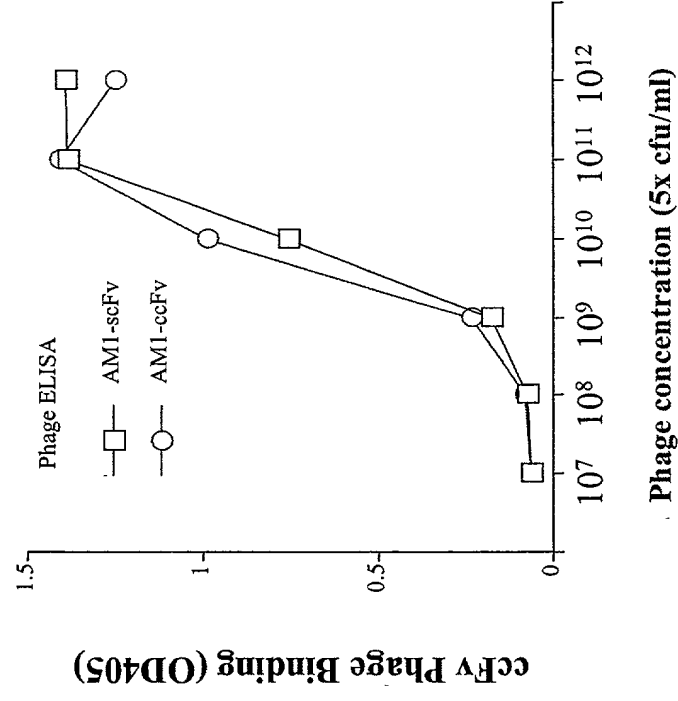


Fig. 11A

Comparison of antigen binding capability of AM2-ccFv and AM2-scFv displayed on phage particles

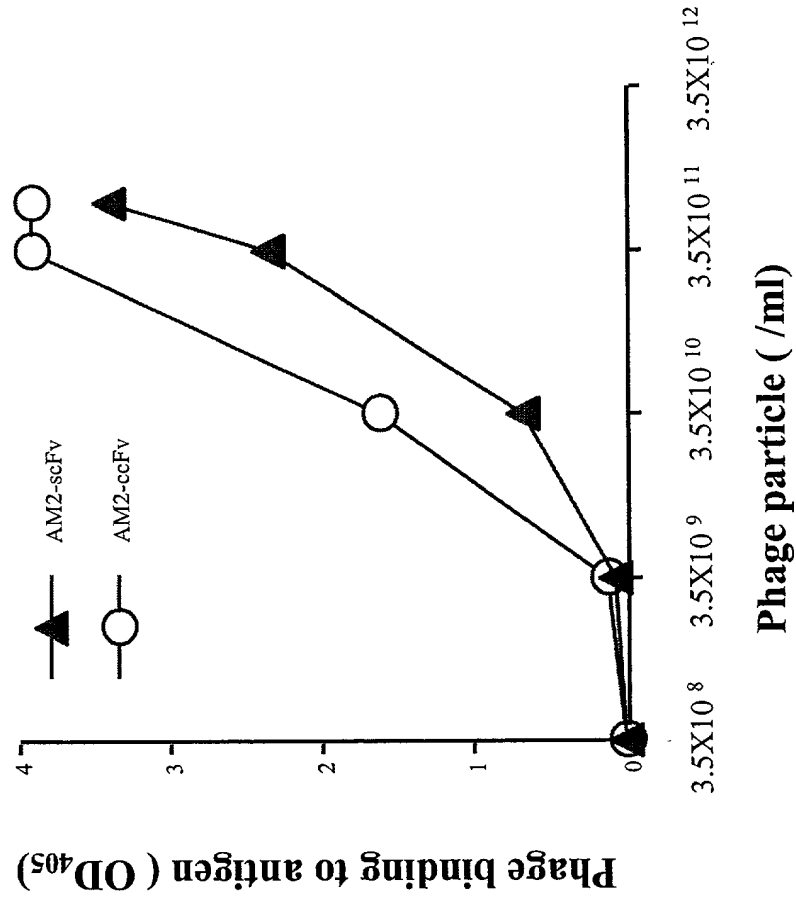
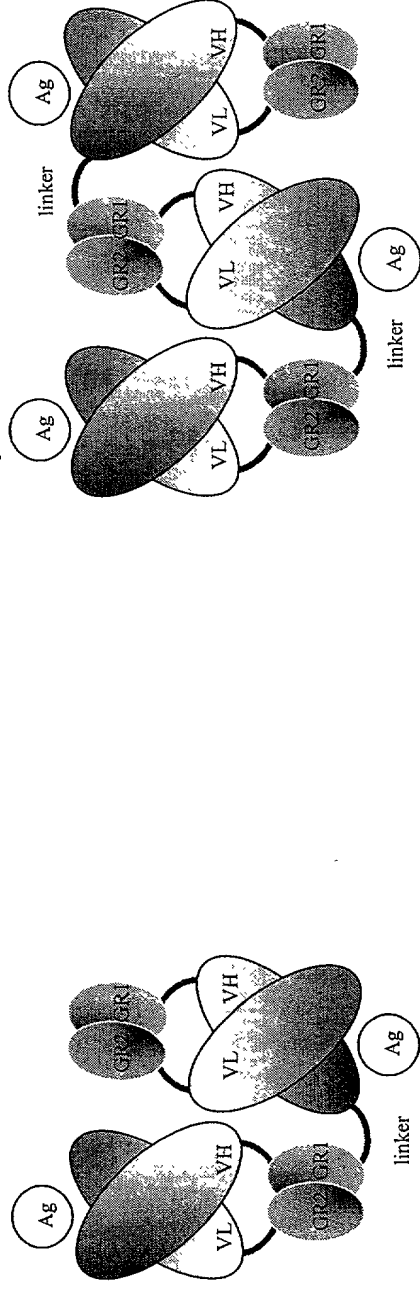


Fig. 11B

Multi-valent ccFv antibody



trivalent (ccFv)₃ peptide 1: VH-GR1-VH-GR1-VH-GR1
peptide 2: VL-GR2

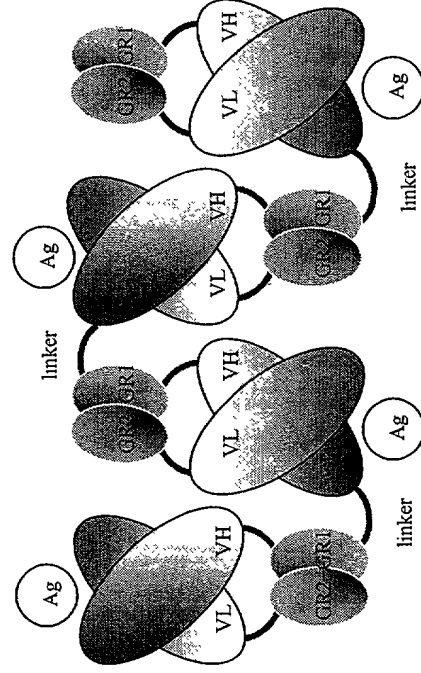


Fig. 12



Fig. 13

Tri-valent ccFv-scFv/dsFv antibody

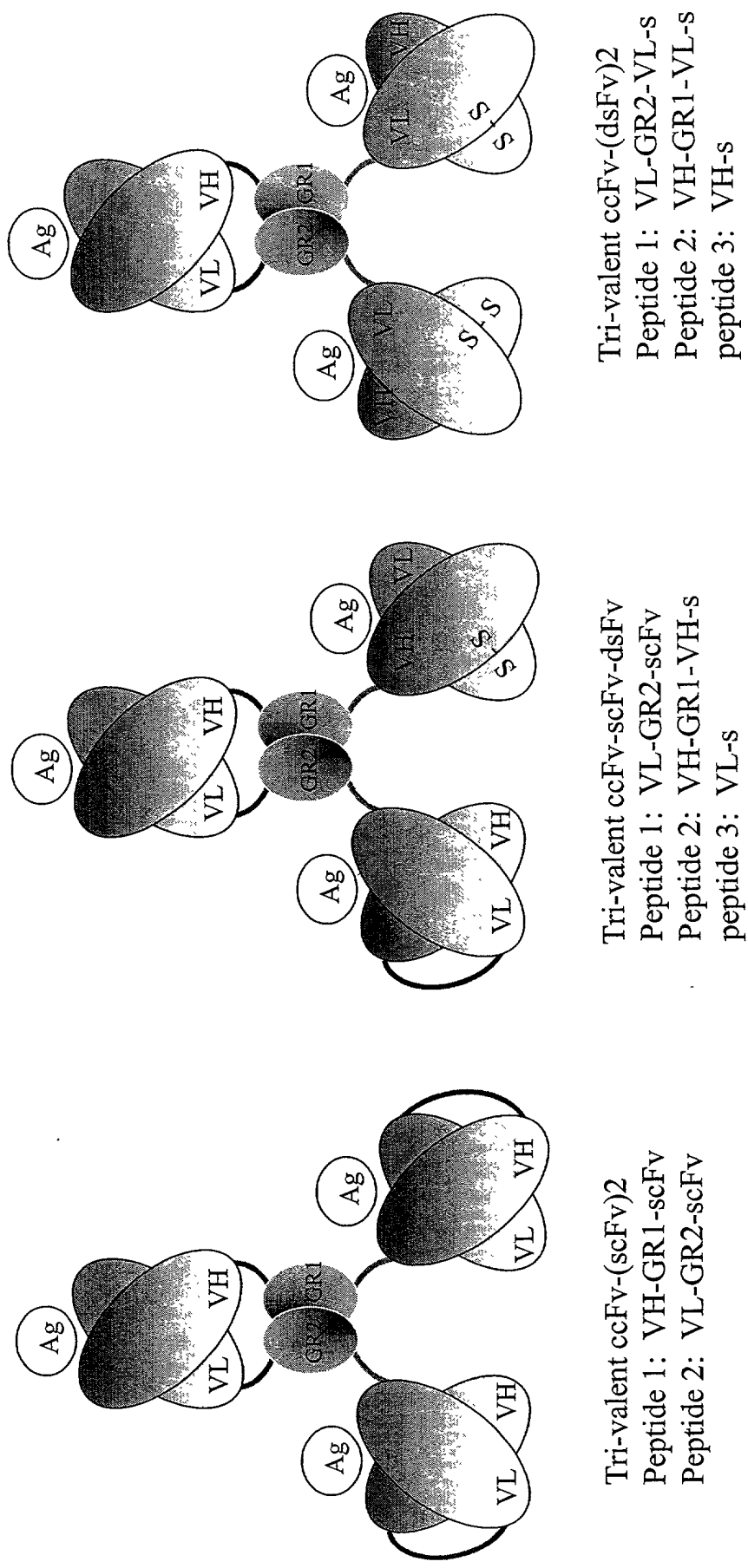
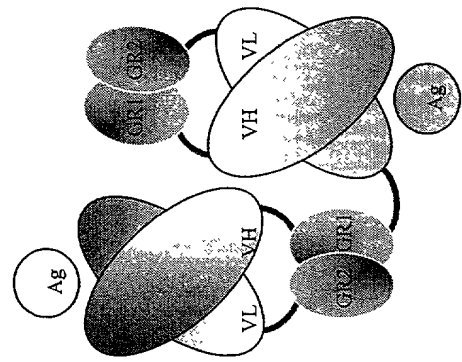
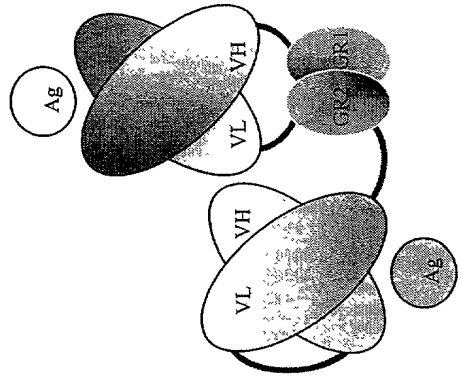


Fig. 14

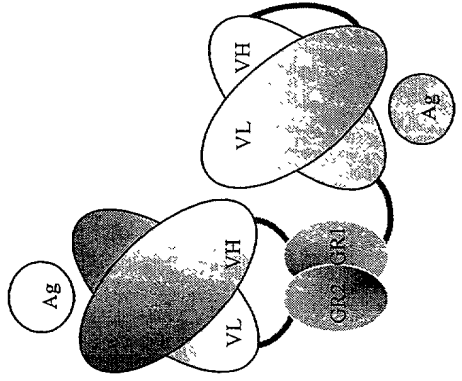
Bi-specific antibody (1)



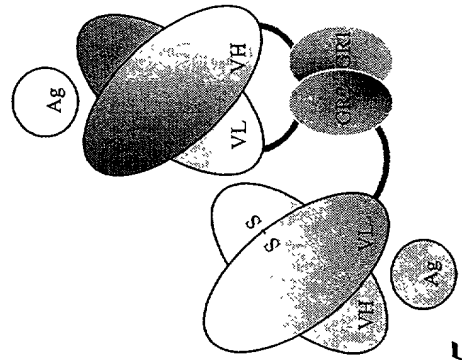
Bi-specific ccFv-ccFv'
 Peptide 1: VH-GR1-VL'-GR2
 Peptide 2: VL-GR2
 Peptide 3: VH'-GR1



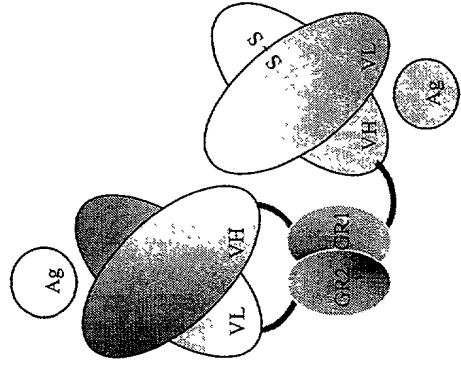
Bi-specific ccFv-scFv'
 Peptide 1: VL-GR2-scFv'
 Peptide 2: VH-GR1



Bi-specific ccFv-scFv'
 Peptide 1: VH-GR1-scFv'
 Peptide 2: VL-GR2



Bi-specific ccFv-dsFv'
 Peptide 1: VL-GR2-VL'-s
 Peptide 2: VH-GR1
 Peptide 3: VH'-s



Bi-specific ccFv-dsFv'
 Peptide 1: VH-GR1-VH'-s
 Peptide 2: VL-GR2
 Peptide 3: VL'-s

Fig. 15

Bi-specific antibody (2)

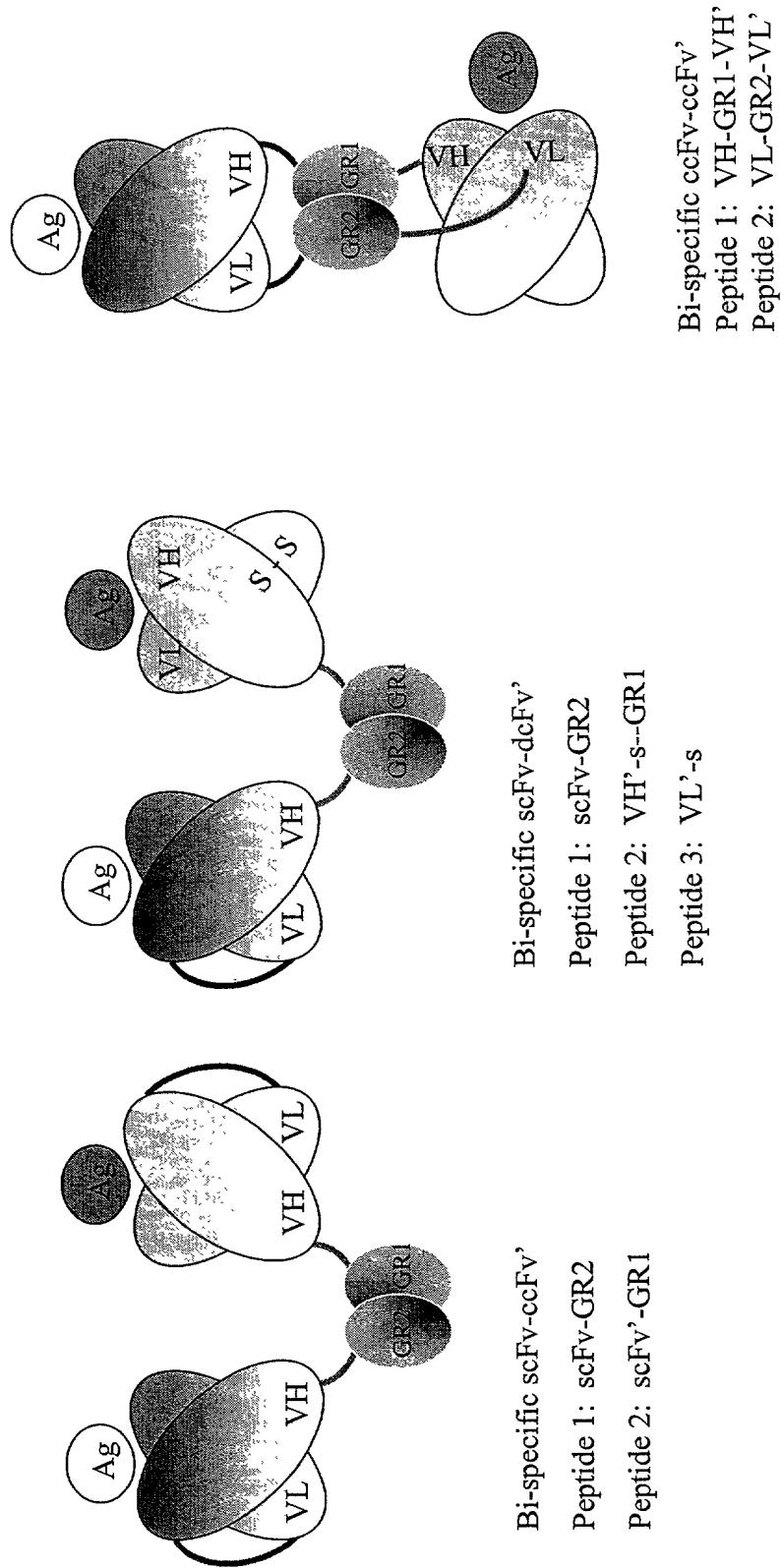
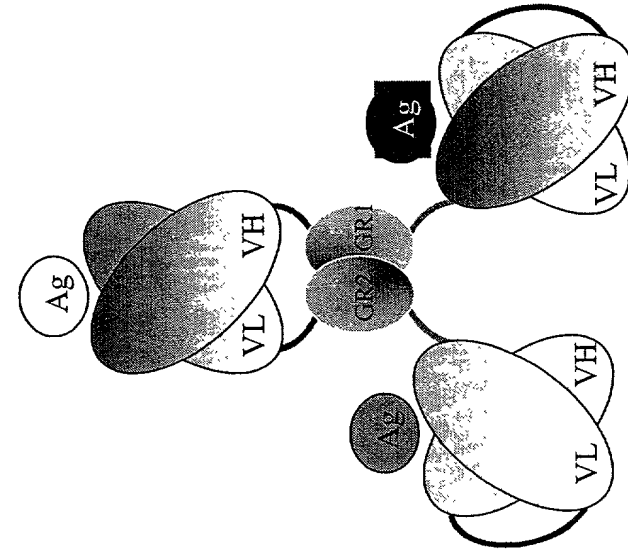
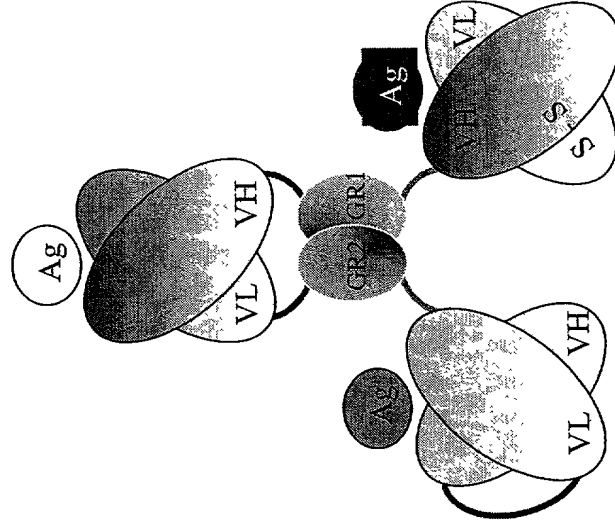


Fig. 16

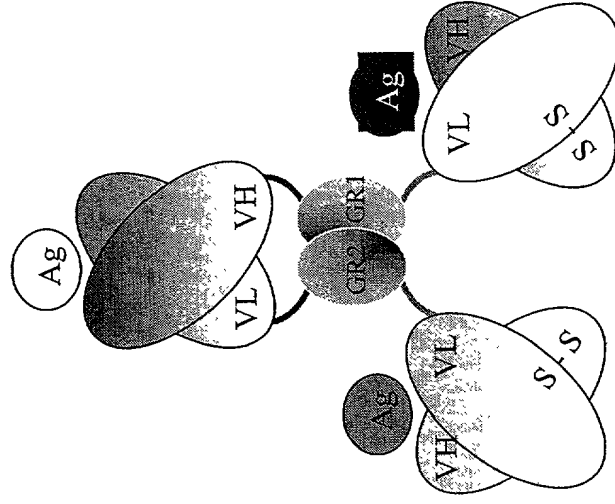
Tri-specific antibody



Tri-specific ccFv-scFv'-scFv*
 Peptide 1: VH-GR1-scFv'
 Peptide 2: VL-GR2-scFv*



Tri-specific ccFv-scFv'-dsFv*
 Peptide 1: VL-GR2-scFv'
 Peptide 2: VH-GR1-VH*-s
 peptide 3: VL*-s



Tri-specific ccFv-dsFv'-dsFv*
 Peptide 1: VL-GR2-VL'-s
 Peptide 2: VH-GR1-VL*-s
 peptide 3: VH'-s
 peptide 4: VH*-s

Fig. 17

Single-chain ccFv antibody

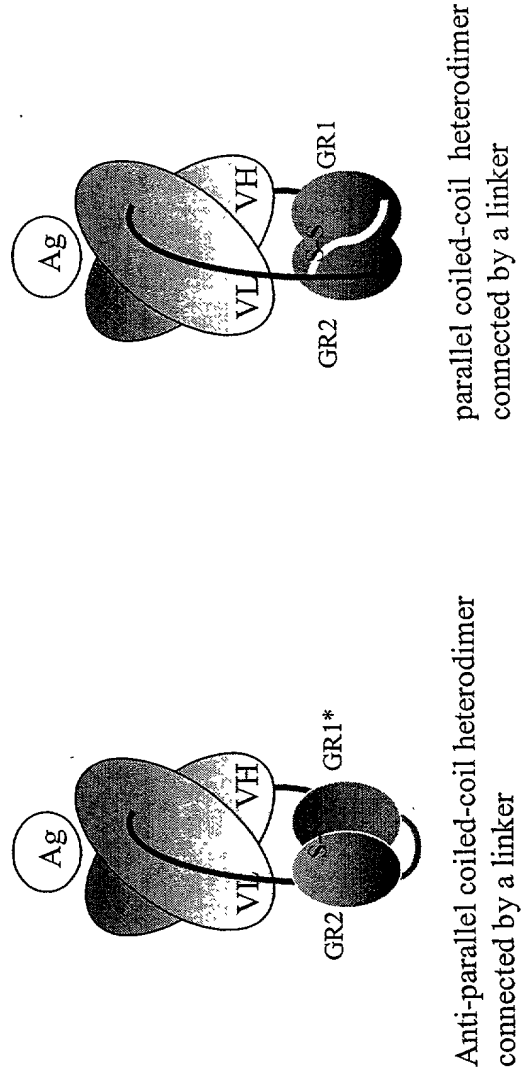


Fig. 18

ccFv display and its use in antibody library construction

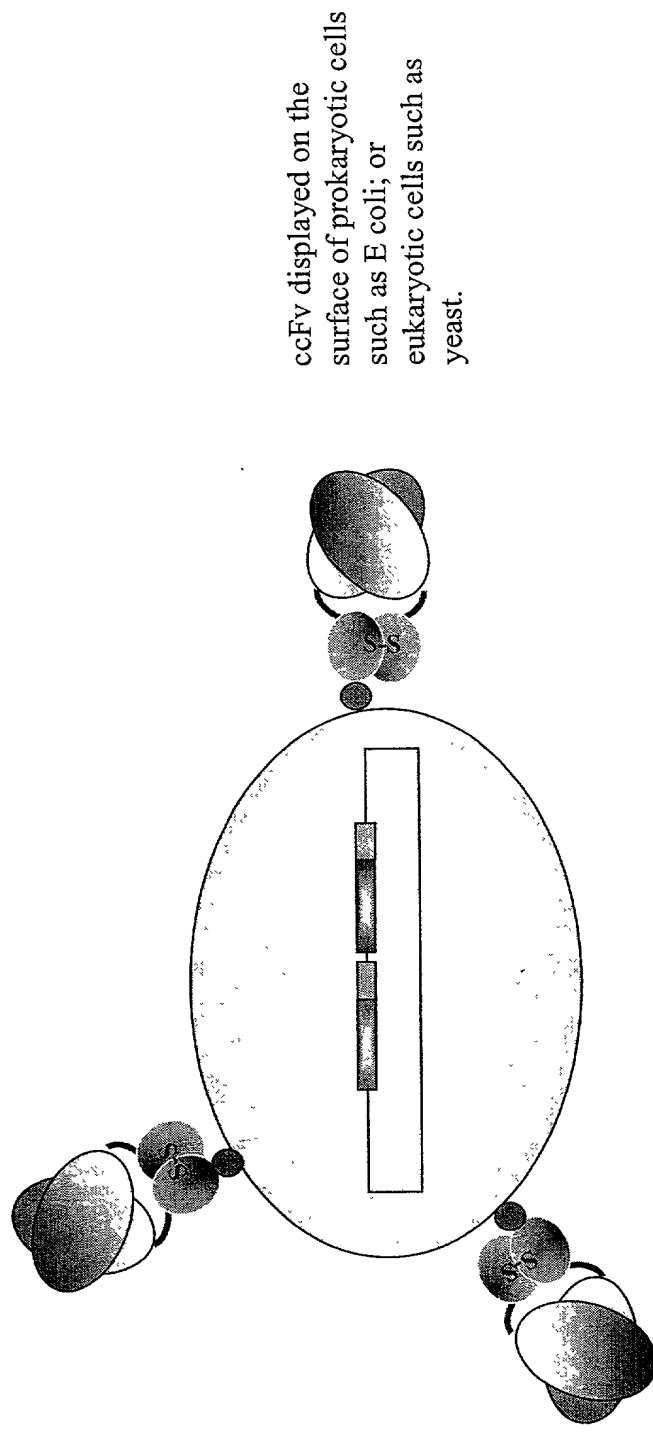
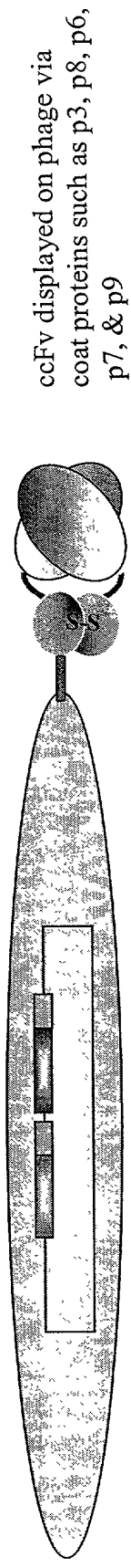


Fig. 19